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Research Article
Association Between NsiI and PmLI Insulin Receptors on the Development of Polycystic Ovarian Syndrome

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Abstract

Background and Objective: Polycystic Ovarian Syndrome (PCOS) is a hormonal abnormality that influences the age during reproduction. This investigation aimed to identify the impact of insulin receptor-encoding genes (NsiI and PmLI) on the development of PCOS and their effect on insulin and HOMA-IR levels. **Materials and Methods:** The study included 80 patients and 25 healthy individuals. The concentrations of HOMA-IR, fasting blood sugar and fasting insulin hormone were determined. The PCR-RFLP was applied to identify insulin receptors in the NsiI and PmLI SNPs. Sanger sequencing was used for each of these patients. The study data were analyzed using SPSS version 16.0 and using χ^2 test $p<0.05$ was considered statistically significant. Also, Hardy-Weinberg equilibrium for genotype frequencies was used. **Results:** The HOMA-IR and mean insulin levels significantly differed between the control subjects and PCOS females ("p = 0.002 and "p = 0.000, correspondingly). Concerning the odds ratio and their NsiI frequency polymorphisms in the heterozygote genotype A/G and homozygote mutant G/G groups were greater in PCOS than control individual (OR = 1.14, p>0.05) (OR = 5.20, p>0.05). However, for the PmLI polymorphism, CC and TT were linked with pathogenic effects for PCOS susceptibility (OR = 1.83, p>0.05) (OR = 12.07, p>0.05) and CT was a protective factor (OR = 0.22, p<0.05). **Conclusion:** A strong relationship between high levels of hormone insulin as well as elevated HOMA-IR has been found in women with PCOS. Furthermore, INSR gene polymorphisms may be a molecular marker associated with decreased insulin sensitivity in women with PCOS.

Key words: Polycystic ovarian syndrome, insulin receptors, polymerase chain reaction, restriction fragment length polymorphism, Sanger sequencing

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is a widespread metabolic disorder recognized for its complicated nature. Multiple studies have shown that this is the most common endocrine disease and genetics play a major role in developing it[1,2]. The clinical manifestations of polycystic ovarian syndrome include hyperandrogenism, impaired ovulation and the presence of ovarian cysts, with metabolic aberrations such as insulin resistance[3]. Its etiology of PCOS remnants was somewhat understood. Despite this, women diagnosed with PCOS involvement many further health complications in addition to unpredictable periods and high testosterone levels[4]. Some of these are a greater risk of type 2 diabetes mellitus, problems regulating glucose, high insulin levels, insulin resistance and being overweight[5]. Insulin resistance is highly prevalent in the majority of obese individuals, ranging from 65 to 90% and is also observed in 25 to 45% of nonobese individuals[6]. This resistance stems from defects occurring in the receptor or post-receptor regions of peripheral cells, such as in skeletal muscle, fibroblasts and subcutaneous fat leading to an inadequate response to insulin. Consequently, the existence of insulin fails to elicit the expected cellular response[7]. Due to impaired insulin response, PCOS patients experience hyperinsulinemia, which is a compensatory mechanism triggered by negative feedback from insulin resistance. Hyperinsulinemia exerts two primary effects, namely, on vascular endothelial cells and ovarian theca cells[8]. Like Luteinizing Hormone (LH), insulin stimulates the manufacture of ovarian steroid hormones, especially ovarian androgens. Insulin causes the liver to produce less SHBG as a sex hormone-binding globulin; thus, the quantity of released androgenic hormones increases[9]. As a result, these two routes combine to activate ovarian theca cells and encourage the generation of more androgens. Persistent oligo- or anovulation and irregular menstrual cycles are caused in part by this disturbance in the regular process of folliculogenesis[10].

Insulin resistance has been linked to obesity in those who suffer from polycystic ovarian syndrome based on multiple clinical investigations[11,12]. Several genes, including those participating in insulin action, were identified as possible biomarkers for PCOS among these genes and INSR is associated with PCOS risk[13]. The INSR genes reside on the 19th chromosome and also encompass 22 exons with 21 introns. Exons 17-21 are specifically important for encoding the tyrosine receptors in the kinase domain. This domain has a crucial function in facilitating insulin signal transduction[14]. Several studies have reported the presence of different polymorphisms within both the coding and noncoding regions (PmLI rs1799817 on exon 17 (C/T) and NsiI rs2059806 on exon 8 (A/G) within the INSR genes in individuals with PCOS that contribute to PCOS risk[13,15]. Therefore, this investigation aimed to examine the relationship between a specific SNPS, PmLI and NsiI and its association with PCOS. Hence, the most important goal of this research was to look at the association between a particular (SNP) rs1799817, situated in exon 17 and NsiI rs2059806, PCOS is around exon 8 of the Insulin receptors genes. Moreover, the objective has been to investigate the possible correlations between genetic variations related to insulin resistance and insulin levels in the bloodstream. In addition, investigated a potential correlation between resistance to insulin concentrations and genetic variations.

MATERIALS AND METHODS

Study participants

Choice of volunteers: The research involved a total of 105 Iraqi women, with 25 (23.8%) classified as healthy subjects and 80 (76.2%) identified as PCOS subjects. These participants were chosen within the Part of (Obstetrics and Gynecology) at a Maternity and Child Teaching Hospital between March, 2023 and September, 2024.

The participants in the study have been divided into two groups with separate categories. The primary categories: Consisted of 80 individuals who were diagnosed with Polycystic Ovarian Syndrome (PCOS). These patients were chosen based on the criteria established by the Rotterdam Criteria[16] , which required having a minimum of two of each of the three stipulated criteria: Ultrasound evidence of polycystic ovarian features, oligomenorrhea/anovulation refers to infrequent or absent menstrual periods, accompanied by clinical or laboratory indications of excessive male hormone levels (hyperandrogenism). Experienced gynecologists confirmed the diagnosis of PCOS and members were included in the study before receiving any treatment. Second, the control group consisted of 25 healthy young women without obstetric, gynecological or internal medical illnesses or fertility concerns. The exclusion criteria for the control group were the use of multivitamin/mineral supplements for two months or the use of hormonal intrauterine devices. Patients who were receiving oral contraception, who had thyroid/renal/liver problems, who had diabetes and who were pregnant or lactating were excluded from the study.

Table 1: Primers used to detect INSR genes in the present study

Genetic variation for INSR	Primer's sequence	Size of the products in (bp)
NSII (rs2059806)	Forward: 5'-CGGTCTTGTAAGGGTAACTG-3'	324
	Reverse: 5'-GAATTCACATTCCCAAGACA-3'	
PmLi (rs1799817)	Forward: 5'-CCAAGGATGCTGTGTAGATAAG-3'	317
	Reverse: 5'-TCAGGAAAGCCAGCCCATGTC-3'	

Ethics approval committee: The research achieved compliance with the ethical principles regarding the Helsinki Declaration, which provides guidelines for conducting medical research relating to members. The study protocol received approval from the Committee for Medical Ethics at the University of Anbar in Ramadi, Iraq, on September 24, 2023 (permit number 141).

Serological assessment: A sterile disposable needle was utilized to obtain 2 mL of peripheral blood by an aseptic technique. Next, 6 mL of blood were placed into a tube that contained a gel clot activator. Centrifugation was performed for 5 min at 1500 rpm to separate the serum. A serological test was conducted to detect serum insulin hormone using a fully automatic ELISA technique (Germany's Elisys Uno Human) with 100 mL of the serum within a normal range (2.00-25.00 µIU/mL). The fasting blood sugar (FBS) measurement was carried out by using an absorbance-based method provided by the Apple Company from Japan. The formula has been used to evaluate the Homeostatic Model[17]:

$$\frac{\text{Fasting insulin (µIU/mL)}\times\text{Fasting glucose (mg/dL)}}{405}$$

Molecular portion of the study

Extraction of genetic material (DNA): Two milliliters of blood were collected in tubes with EDTA to examine INSR polymorphisms. A total of 400 µL of drawn blood was used to extract the genomic DNA using SaMag's kit for separating DNA from blood tests and the SaMag-12 automatic genetic material extraction equipment from (Sacace Biotechnology in Italia). This study followed established protocols for extraction[18].

The process of extracting DNA from blood involves several consecutive phases, which include lysis, elution, washing and binding. A Quantus™ fluorometer and a QuantiFluor® double-stranded technique (Promega, the United States; Madison, Wisconsin) were applied to evaluate the level of concentration of the isolated DNA. An ultraviolet spectrophotometer, Thermofisher, UK was used to measure the OD260/OD280 ratio to determine the purity of the DNA[19].

PCR technique for specific DNA sequence amplification: The PCR equipment from ESCO, USA, Riverside, CA, was used to amplify segments of DNA and an order was placed with a Macrogen Company for the necessary primers. In the initial stage, a stock solution was prepared. This was accomplished by lyophilizing and dissolving the primers in water without nuclease until the desired concentration of 3.00 pmol/L was reached. The Green Master Mix, available via Promega in the United States, was used for PCR. The 3 µM $MgCl_2$, 400 µM (dATP, dGTP, dCTP and dTTP) and Taq DNA polymerase were included in the master mix. These components were all contained within a reaction reagent with a pH of 8.5. The experiment was performed by using a solution consisting of 25 µL, which contained (12.5 master mix and 1 µL) each of forward and reverse primers. The quantity of target DNA was 3 and 7 µL of nuclease-free water had been added. To amplify the DNA segment, PCR which carried out under the given circumstances. There were 35 PCR cycles for each gene, with initial denaturation being the first step (5 min at 95°C). Afterward, denaturation was carried out for 45 sec at 93°C, then annealing for 30 sec at 56°C for NsiI and PmLI. In the following 45 sec, the extension processes were conducted at 72°C. The last stage of the extension was completed after 5 min. The last extension stage was accomplished for 5 min at 72°C. The conducting power source had been connected to the electrodes (for 5 min at 50 volts). Subsequently, the voltage was increased to 100 and kept up for an hour. Subsequently, it looked into the DNA bands under the light of a transilluminator with Ultraviolet (UV) radiation manufactured by Vilber Lourmat in the city of Lemont, Illinois, USA. An investigation was conducted on PCR products with diameters of 324 bp (NsiI) and 317 bp (PmLI) (Table 1).

Investigated NsiI (rs2059806) variant through Restriction Fragment Length Polymorphism (RFLP): The NsiI-restricted enzymes originating from Bio Labs in the USA were utilized to digest the PCR products of the INSR gene, which had a length of 324 base pairs. The NsiI digestion process lasted for 3 hrs at 37°C and NsiI was subsequently allowed to react for 20 min at 65°C. To analyse the PCR products that were digested by enzymes, agarose gels containing a 1.5% concentration were used. Agarose gels (1.5%) were used, which were prepared using ethidium bromide in a 1X TBE buffer solution. Electrophoresis was carried out for 60 min at 100 volts. Digesting of NsiI resulted in homozygous wild-type A/A (324 bp). Homozygous mutants of G/G were 239 and 85 bp long. The heterozygous A/G ratios were 324, 239 and 85 bp.

Investigated PmLI (rs1799817) variant through Restriction Fragment Length Polymorphism (RFLP): The PmLI-restricted enzyme from Bio Labs in the United States, which has a length of 317 bp, was employed to break down the PCR products of the PmLI gene. The PmLI breakdown process lasted for 3 hrs at 37°C and was subsequently stopped for 20 min at 65°C. To analyze end results of PCR that were digested by enzymes, 1.5% agarose gels were used, which were prepared with ethidium bromide in a 1X TBE buffer solution. Electrophoresis was carried out for 60 min at 100 volts. Digestion of PmLI results in homozygous wild-type C/C. The homozygous mutants of T/T were 271 and 43 bp long. The heterozygous C/T ratios were 317, 274 and 43 bp.

Sequencing: An ABI3730XL platform equipped with Sanger dideoxynucleoside sequencing technology was utilized to detect polymorphisms in the study samples. This platform was supplied by the Macrogen Company in Seoul, Republic of Korea Province. The purpose of sequencing was to determine whether the sequenced genotypes matched those produced by PCR-RFLP technique as declared and observed by Abdulrazaq *et al.*[19].

Analysis of data: For the variable analysis, IBM Corporation, Armonk, New York, USA, provided SPSS 22 software. The examiners performed an independent t-test for comparison of the categories that were analyzed. A level of statistical significance indicated as $p<0.05$ was utilized to establish significance. The WinPepi statistical program, version 11.65, has been employed to figure out if the p-values generated by the Fisher test were statistically significant. In addition, a specific χ^2 computation was used to evaluate the odds ratio.

RESULTS

Studies including biochemical and serological markers in females with PCOS and healthy subjects: Approximately 80 individuals suffering from PCOS and 25 control individuals participated in this study. The study revealed that the mean fasting insulin levels were 18.657±16.93 for individuals with PCOS and 3.56±1.38 for healthy subjects. Notably, there were significant variations in the average insulin levels of the PCOS patients. The patients and healthy group showed a statistically significant variation ($p = 0.00$). Furthermore, the mean FBS levels were 91.13±11.18 for PCOS patients and 83.680±5.764 for the control group. Significant differences were not found among the two categories ($p = 0.59$) in the research. Additionally, to confirm the presence of resistance to insulin, the homeostasis modelling assessments of HOMA-IR value were analyzed. The HOMA-IR levels equal to or greater than 2 were classified as insulin resistance, while HOMA-IR levels below 2 were classified as not having insulin resistance. The average Insulin resistance of PCOS women amount was 16.495±108.17, while in the control group, the mean VD was 0.71±0.28 ($p = 0.00$).

Molecular study parts

Correlations between INSR (NsiI) gene polymorphisms and PCOS: Findings for the PCR-amplified gene DNA fragment the PCR products were then exposed to RFLP analysis, through the ends of the reactions visible on an ethidium bromide-stained 1.5% agarose gel. The RFLP analysis results for three genetic types (AA, AG and GG) were shown in Fig. 1a-b.

The distributions of the frequency of alleles and genotypes for the NsiI (rs2059806) SNP in exon 8 (A/G) were presented in Table 3 for the groups according to the study. The controls were in agreement with the HWE and there was a significant correlation between disease severity and genotype AA (OR 0.61; CI 95% (0.23-1.56)). In individuals carrying the (AG and GG) homozygosity genotypes, the results presented among PCOS and control categories, there were no statistically significant differences. However, these genotypes showed a positive relationship with the condition, as determined by the odds ratio (OR) (OR 1.14; 95% CI between 0.44 and 2.982 for the heterozygosis AG genotype and odds ratio 5.20; 95% CI between 0.29 and 94.39 for the homozygosity GG genotype). Moreover, the odds ratio (OR) associated with the A allele was 0.53. Therefore, a negative correlation with the disease, as indicated by the OR, might be interpreted as a genetic variant that protects against PCOS. The OR for the G alleles was 1.87, which suggests a positive relationship with PCOS and could be regarded as a causative factor that potentially increases the risk for Iraqi women. The distribution of NsiI SNPs under the genotyping model in the study population is shown in the same table and there were no significant differences (all p values greater than 0.05). No significant correlation was detected under either the dominant or recessive models as represented in Table 3 and Fig. 2.

Table 2: Comparison of serum fasting insulin, FBS and HOMA-IR among PCOS patients and healthy individuals

Parameter	Insulin	FBS	HOMA-IR
Patients (mean±SD)	18.66±16.93	91.13±11.18	16.49±108.17
Control (mean±SD)	3.56±1.38	83.68±5.76	0.71±0.28
p-value	0.00	0.59	0.00

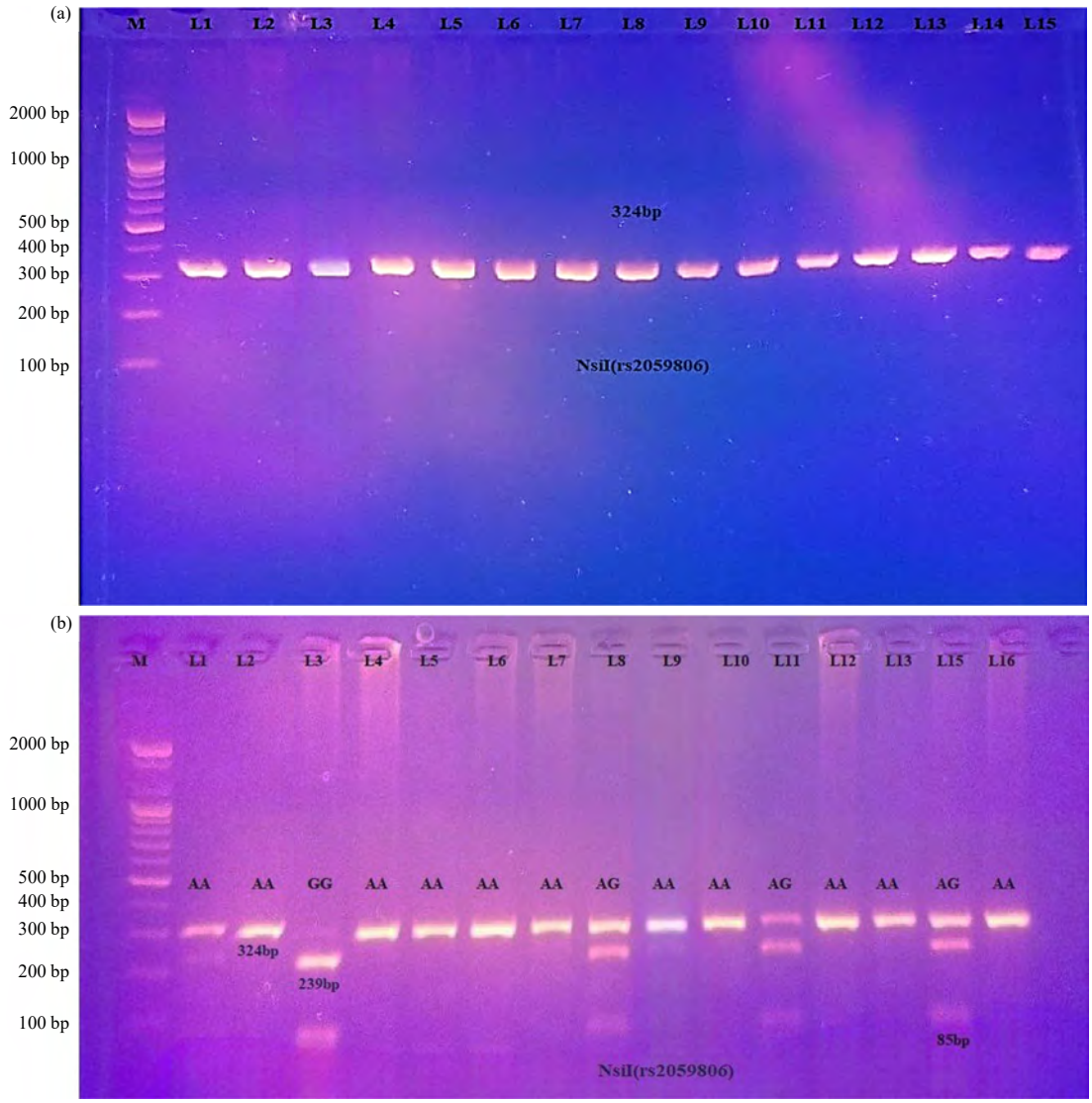

Fig. 1(a-b): (a) Agarose gel electrophoresis (1.5%) stained by ethidium bromide and (b) Electrophoretogram of DNA fragments for NsiI polymorphisms after digestion with NsiI restriction enzymes

(a) PCR product for INSR gene (NsiI) with an expected size 324 base pair and (b) PCR-RFLP results of a patient revealing the presence of homozygous wild-type bands represented 324 base pairs A/A. The 239- and 85-base pair bands represent the homozygous G/G mutant. Heterozygous A/G was revealed by the bands of 324, 239 and 85 base pairs

Table 3: Frequency analysis and HWE of genotypes, alleles and genetic models for the rs2059806 SNP of the INSR (NsiI)

	Frequency (n (%))					
Genotype	PCOS	Control	HWE	p-value	Odds ratio	CI 95%
AA	45 (56.25%)	17 (68%)	p = 0.34	0.29	0.61	0.23-1.56
AG	28 (35%)	8 (32%)		0.78	1.14	0.44-2.98
GG	7 (8.75%)	0 (0.00%)		0.27	5.20	0.29-94.39
Allele distribution						
A	118 (73.75%)	42 (84%)	χ^2 =0.907	0.14	0.53	0.23-1.23
G	42 (26.25%)	8 (16%)		0.14	1.87	0.81-4.30
Genetic models						
AA	45 (56.25%)	17 (68%)		0.29	0.60	0.23-1.5670
AG/GG	35 (43.75%)	8 (32%)				
AA/AG	73 (91.25%)	25 (100%)		0.26	0.19	0.011-3.48
GG	7 (8.75%)	0 (0.00%)				

Significant difference is at $\leq$0.05, OR: Odds ratio, CI: Confidence interval and Statistically by Fisher test

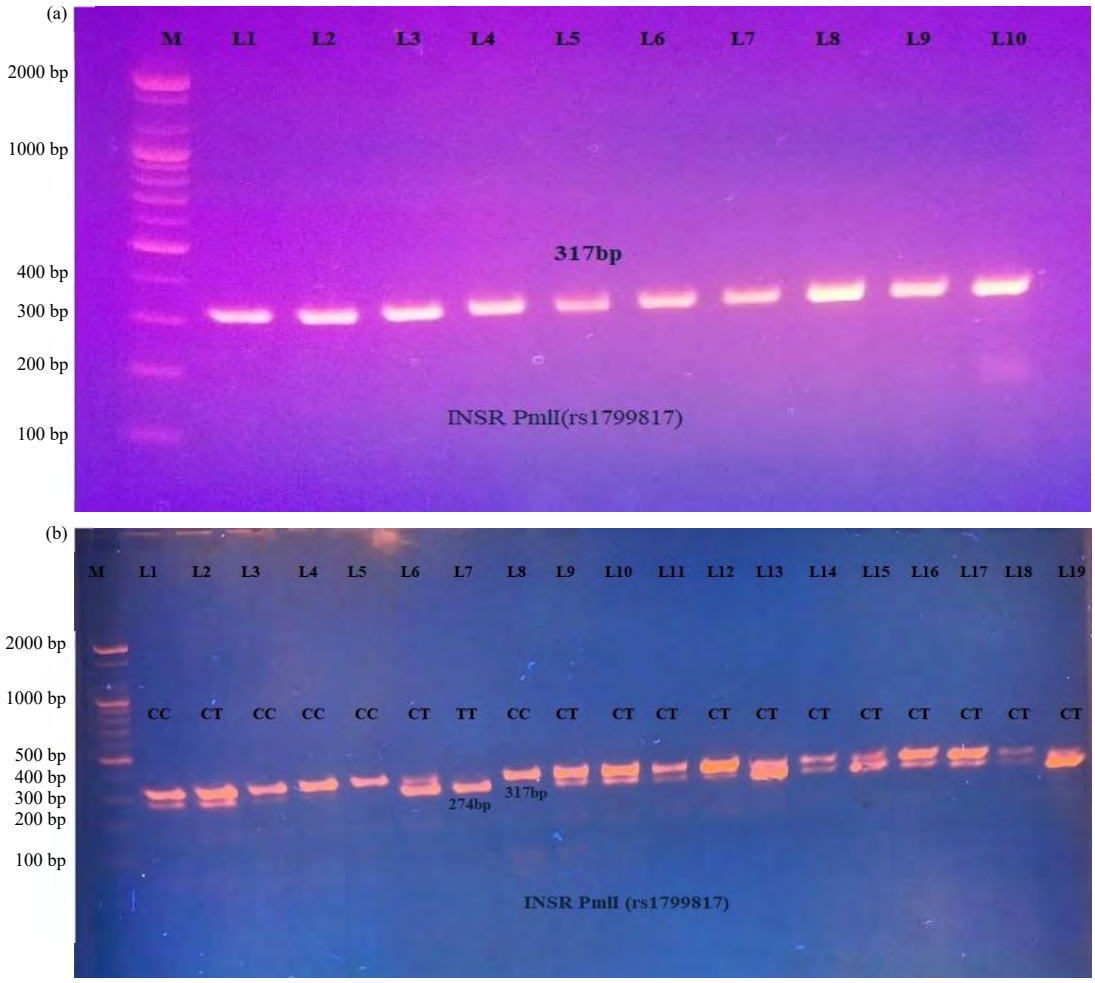

Fig. 2(a-b): (a) Agarose gel electrophoresis (1.5%) stained by ethidium bromide and (b) Electrophoretogram of DNA fragments for PmLI polymorphisms after digestion with PmLI restriction enzymes

(a) PCR product for INSR gene (PmLI) with an expected size 317 base pair and (b) PCR-RFLP results of a patients: The homozygous wild-type bands presented 317 C/C base pairs. A total of 271 and 43 base pairs were presented with the homozygous mutant bands T/T, respectively. Heterozygous bands were revealed by 317, 274 and 43 base pairs C/T. Note that the 43 base pair bands were separated from the gel

Correlations between INSR (PmLI) gene polymorphisms and PCOS: The DNA segment of this gene was amplified by PCR. After that, the PCR products were subjected to RFLP analysis, as well as the results were investigated on a 1.5% agarose gel that had been colored with ethidium bromide. The results of the RFLP analysis of the three genetic variants (A/A, A/G and G/G) were displayed in Fig. 2a-b.

The distribution of INSR (PmLI) polymorphism alleles and genotypes in PCOS patients. The distribution of the INSR SNP in the healthy individual HWE equilibrium was significant ($p = 0.000085$), possibly due to the stratification of the control sample and its association with PCOS risk.

The CT genotyping was the most prevalent genotype in the control group populations, whereas in the patient groups, the CC and TT genotypes were the most predominant. However, when comparing healthy women with PCOS, a significant CT heterozygous gene was detected ($p = 0.02$), indicating a negative correlation with PCOS (OR 0.22; CI 95% (0.06 and 0.78)). Furthermore, in PCOS patients and healthy controls, there were no significant changes, in the homozygous CC and TT genotypes were identified, but the odds ratios were positively correlated with medical conditions (OR 1.83; 95% CI 0.49-6.89 and 12.07; 95% CI 0.69-299.29, respectively). In addition, the OR for allele C was 0.53, with a CI of 0.23-1.23. Additionally, the OR for allele T was 1.87, with a CI of 95% between 0.81 and 4.30, showing a positive relationship with medical conditions, as shown in Table 4. An important difference was found in the results of this study, in the combined C/T-C/C genotype under the recessive model ($p = 0.02$) as shown in Table 4 and Fig. 3.

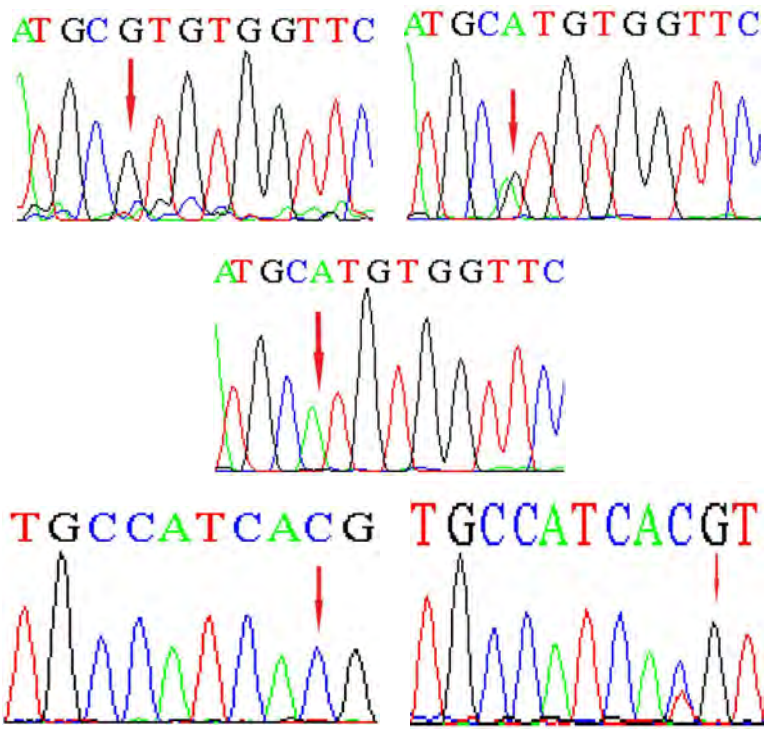

Fig. 3: DNA sequencing of NsiI (AA, AG and GG) in the upper section and PmLI (CC and CT) in the lower section

Table 4: Frequency analysis and HWE of genotypes, alleles and genetic models for the rs1799817 SNP of the INSR (PmLI)

	Frequency (n (%))					
Genotype	PCOS	Control	HWE	p-value	Odds ratio	CI 95%
CC	16 (20%)	3 (12%)	p = 0.00	0.37	1.83	0.49-6.89
CT	49 (61.25%)	22 (88%)		0.02	0.22	0.06-0.78
TT	7 (18.75%)	0 (0.00%)		0.08	12.07	0.69-299.29
Allele distribution						
C	81(50.63%)	28 (56%)	χ^2 =15.43	0.51	0.81	0.43-1.53
T	79 (49.38%)	22 (44%)		0.51	1.24	0.65-2.35
Genetic mode						
CC	16 (20%)	3 (12%)		0.64	1.83	0.48-6.89
CT/TT	64 (80%)	22 (44%)				
CT/CC	65 (81.25%)	25 (100%)		0.02	23.74	0.39-403.10
TT	30 (37.5%)	0 (0.00%)				

Significant difference is at ≤0.05 and HWE: Hardy-Weinberg Equilibrium

Table 5: Stratification analysis of the impact of INSR genetic polymorphism on serum levels of fasting insulin level

INSR/SNPs	Genotype	Patient (Mean±SD (ng/mL))	Healthy (Mean±SD (ng/mL))	p-value
NsiI rs2059806	AA	16.6±17.7	3.6±1.4	0.004
	AG	20.8±16.2	3.4±1.3	0.005
	GG	18.02±16.06	-	-
PmLI rs1799817	CC	20.1±17.2	3.2±.92	0.115
	CT	17.1±13.6	3.6±1.4	0.000
	TT	19.8±25.7	-	-

Mean significant difference is at ≤0.05

Impact of INSR genetic polymorphism on serum level of fasting insulin level: Insulin hormone may show an active contribution to the pathogenicity of PCOS. The INSR has SNPs that may have positive or negative effects associated with PCOS. There are three genotypes (AA, AG and GG) for NsiI (rs2059806 A/G) and (CC, CT and TT) for PmLI (rs1799817C/T) as represented in Table 5 which reflects the genetic variation in these two sites and the effect on the mean serum level of insulin.

NsiI rs2059806 A/G: Data was revealed for the first site of NsiI (rs2059806 A/G). It has three genotypes with a highly significant impact on the serum level of insulin. It has been observed that the homozygous (AA, GG) genotype had a positive impact and increased serum levels in patients compared with the healthy control (16.6±17.7 ng/mL) vs (3.6±1.4 ng/mL), consequently with p-value = 0.004. In GG genotype, (18.02±16.06 ng/mL) of the patients revealed the presence of this genotype with the absence of it among the healthy subjects. Furthermore, the AG genotype had a too positive impact and raised serum amount in patients (20.8±16.2 ng/mL) compared to healthy control (3.4±1.3 ng/mL), p = 0.005.

PmLI rs1799817C/T: The second site of PmLI rs1799817C/T was notable, CC genotype was scored 20.1±17.2 vs 3.2±.92 pg/mL, respectively with p = 0.115. Moreover, it has been observed that CT genotype had a positive impact on serum level of insulin and an increase of serum levels in patients compared with the healthy control 17.1±13.6 vs 3.6±1.4 ng/mL consequently. In addition, the presence of the TT genotype (19.8±25.7ng/mL) with the absence of it in the control group was highly observed.

DISCUSSION

This cross-sectional study revealed a significant increase level of insulin while this was not observed with the fasting glucose between PCOS women and healthy control. The result was in agreement with those observed by Akram *et al.*[20], as they reported that people with PCOS and those in the control group did not significantly differ in their fasting glucose levels; it was also found that individuals with PCOS revealed significantly elevated fasting insulin levels ($p<0.05$) linked to those in the control group. Constant with the results reported by Taghizadeh *et al.*[21], FBS amounts were within normal ranges for both the patients and controls and there were no significant differences among both groups. Furthermore, the concentrations of the hormone insulin were considerably increased in the patient subject than in the control ($p = 0.03$). Additionally, HOMA-IR, an indicator of insulin resistance, had been significantly greater in the patients, suggesting the presence of insulin resistance. Furthermore, the present study data was in agreement with those of Mather *et al.*[22], who reported that patients with polycystic ovarian cancer and hyperinsulinemia had more insulin resistance than controls and the levels of glucose did not differ significantly. An explanation for the observed phenomenon is that hyperinsulinemia, marked by higher levels of insulin in the bloodstream, stimulates the enzyme activity of cytochrome P450c17α in the ovaries of PCOS females. This increased activity leads to increased concentrations of androgens in the bloodstream. Consequently, elevated androgen levels may cause a decrease in the concentration of serum SHBG[23]. Current investigation insulin levels were considerably greater in PCOS-affected women than in control subjects, even though fasting blood sugar levels among people with ovarian polycystic syndrome and controls were within normal limits. Taken together, these findings suggest that insulin secretion increases to compensate for insulin resistance and maintain glucose levels within the normal range. At the same time, the study disagreed with Rashidi *et al.*[24]. The case group reported significantly higher standard deviations of fasting blood glucose and insulin levels.

The INSR gene exhibits several genetic variations and has been linked to PCOS and insulin resistance (IR)[25]. Insulin resistance has been implicated in the upregulation of LH secretion in the pituitary gland and the enhancement of testosterone production in theca cells. These physiological changes can disrupt follicular maturation, potentially leading to the development of PCOS and INSR allelic variants and possibly causing genetic susceptibility to the development of PCOS[26]. In the present study, regarding the odds ratio, we found that the "AG and GG" genotypes and the G allele showed a correlation with heightened risk factors for PCOS susceptibility. The present investigation cannot agree with a prior report conducted by Tehrani *et al.*[27], on Iranian women; there was no correlation between PCOS incidence and the rs2059806 variant. In addition, in a study performed by Feng *et al.*[28], on Chinese females, there appears to be no significant association between PCOS exposure and rs2059806 SNPs based on a meta-analysis.

Additionally, Bagheri *et al.*[29], in this first study, found no correlation between NsiI (rs2059806) SNP in the INSR gene and Iranian Azeri individuals at risk for PCOS Turkish females. This may be because of certain limitations, including incomplete data regarding the tested genes, a small sample size and insufficient quality of registry data. Additional research using large sample sizes and including data from other genetic variants. The PCR-RFLP revealed a Homowild CC genotype and the TT genotype of PmLI (rs1799817), which exhibited the highest frequency among women who have PCOS and is, therefore, the most prevalent. Furthermore, research has demonstrated that the T allele is a main causative factor linked with the development of PCOS in females, based on the odds ratio. Additionally, combining C/T-C/C in a recessive model has been a significant harm effector associated with PCOS in Iraqi women. Current findings agreed

with those of a study in Iraq Babylon via[30], in which the prevalence rates of the T allele and TT genotype were notably greater in PCOS patients than in controls. Among PCOS patients, 31 (62%) but only 22 (42%) of the controls had an odds ratio of 2.25 (1.01-5.01), similar to the findings of studies performed by Siegel *et al.*[31], Kanaan *et al.*[32] and Mutib *et al.*[33]. This finding disagreed with another study by Lee *et al.*[34] which reported no associations between the variables in the Korean population. The observed differences in results between studies can potentially be attributed to various factors, such as ethnic disparities, lifestyle variations and environmental variables throughout the study groups. The PmLI SNP is located within the INSR gene's 17th exon, specifically at the site where ATP binds to the INSR β-subunit containing a tyrosine kinases region. This SNP induces an alteration that is a missense mutation, resulting in the substitution of cysteine with arginine present in two different allele genotypes. The observed correlation is not due to alterations in the production or release of the INSR β-subunit but rather is likely caused by the impact of this single nucleotide polymorphism impact of SNPs on the functionality of the INSR β-subunit. The identified mechanism underlying the link of the SNP with PCOS seems very probable, particularly about reduced sensitivity to insulin in PCOS-affected females[35].

The current study assumed a positive impact of INSR genetic polymorphic in two positions (rs2059806 A/G) and (rs1799817C/T) on the serum level of insulin associated with PCOS. The current study was consistent with Khan *et al.*[36], who mentioned numerous variations. Numerous researchers have examined conditions like type 2 diabetes mellitus and insulin in the INSR gene's exon 17 domain. In an additional investigation by Daghestani[13] were showed PCOS patients with CT and TT genotypes have higher insulin levels. However, a study conducted in Korea did not find any evidence of such relationships, presumably due to differences in ethnicity. There was no notable disparity in insulin and glucose levels among PCOS women with CC and CT genotypes. However, CT and TT genotypes exhibited a significant association with both insulin and glucose levels ($p = 0.002$, 0.007, respectively)[34]. Women with PCOS commonly have reduced sensitivity to insulin. The insulin binding to the INSR gene's α subunit increases the receptor's tyrosine kinase activity, which starts cellular signaling pathways. Females who have been diagnosed with PCOS have skeletal muscle and adipose tissue signaling abnormalities in INSR[13]. As a future recommendation, it is suggested that it is important to perform a detailed study with a larger sample size and include additional confounding parameters on the genetic level.

CONCLUSION

A strong association was seen between the amount of fasting insulin in the blood serum and the values of HOMA-IR in patients with PCOS. Fasting insulin levels and HOMA-IR levels proved to be closely linked. Furthermore, this meta-analysis provides statistical evidence that INSR polymorphisms are correlated with PCOS risk in Iraqi women and are associated with insulin resistance. Polymorphisms of the INSR gene, rs2059806 (exon 8, A/G) and (rs1799817C/T) may be associated with fasting insulin level PCOS females.

SIGNIFICANCE STATEMENT

The purpose of the study was to identify the insulin impact on receptor-encoding genes (NsiI and PmLI) on PCOS development and their effect on insulin and HOMA-IR levels. The objectives include the detection of the relationship between a specific SNPS, PmII and NsiI and its association with PCOS through the detection of the associations between a particular (SNP) rs1799817, situated in exon 17 and NsiI rs2059806, PCOS in exon 8 of insulin receptors genes. Moreover, to investigate the possible correlations between genetic variations encoding insulin resistance and insulin levels. The potential correlation between insulin resistance and genetic variations was highly observed creating the idea that INSR gene polymorphisms may be a molecular marker associated with decreased insulin sensitivity in women with PCOS.

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